Capabilities of Direct Sample Introduction–Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry to Analyze Organic Chemicals of Interest in Fish Oils

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Most analytical methods for persistent organic pollutants (POPs) focus on individual groups of targeted analytes. Therefore, analysis of multiple classes of POPs typically entails several sample preparations, fractionations, and injections, whereas other chemicals of possible interest are neglected or lost. To analyze a wider scope of organic contaminants in fish oil, we developed an approach to combine the analysis of targeted and untargeted chemicals using an automated direct sample introduction (DSI) and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/ToF-MS). DSI-GC×GC/ToF-MS is a powerful tool that attains high quality separations to achieve high selectivity while still providing a wide analytical scope with minimal sample preparation, especially in conjunction with DSI's high tolerance to dirty extracts. Gel permeation chromatography (GPC) was used for initial separation of lipids from POPs and other GC-amenable organic compounds from dietary cod liver oil. For comparison purposes, additional cleanup of the GPC extracts was done by silica adsorption and acidification, which helped provide clues in the identification of untargeted compounds, but in routine analysis, only GPC is needed for this analytical approach. The approach allowed simultaneous identification of known-POPs in the fish liver oils, and further permitted presumptive identifications of multiple groups of halogenated natural products (HNPs) and other organic chemicals of interest through comparisons of the mass spectra from analyses with those from mass spectral libraries and/or reports in the literature (~60 PCB congeners and 76 compounds in total). Subsequent confirmations were made by reanalysis and comparison of chromatographic retention times and mass spectra with contemporaneously analyzed reference standards. Otherwise, ion fragmentation patterns of unknown compounds were assessed for tentative identifications. Some of the HNPs in the fish oils were detected and identified for the first time. Our study demonstrates that the wide monitoring scope provided by the DSI-GC×GC/ToF-MS method after GPC provides many logistical and performance advantages over the conventional use of several different methods designed for individual classes of targeted analytes after extensive sample preparation.

Introduction

Persistent organic pollutants (POPs) bioaccumulate, travel over long distances, and contaminate remote areas. Most POPs contain chlorine, and traditional POPs include poly-chlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs). Some compounds of emerging concern contain bromine, such as the polybrominated diphenyl ethers (PBDEs), which have POP-like properties. National and international monitoring programs continue to analyze these POPs in several environmental compartments, such as air, sediment, fish/wildlife, and humans.

Due to the complexity of the matrices, ultratrace concentrations involved, and issues of selectivity in analysis, most analytical methods for environmental monitoring were specifically developed for a certain group of chemicals, i.e., targeted analysis. However, targeted approaches can miss unknown or other untargeted chemicals in samples, even if their environmental levels may be high. For example, PBDEs and Dechlorane Plus were discovered in environmental matrices well after they were already ubiquitous in the environment (*1–3*).

Because of the targeted analytical methods used for environmental monitoring, pro-active screening approaches have recently been suggested and studied. For example, environmental fate models have been used to screen hundreds of thousands of chemicals registered in a chemical database for their potential to act like POPs (*4, 5*). This approach requires new analytical methods to cover such a wide range of chemicals (*4*). Furthermore, the ability to detect metabolites and environmental degradation products that do not appear in the database is critical. Therefore, more informative, more sensitive, more selective, and faster analytical methods are needed for more efficient monitoring of POPs, POP-like compounds, and other chemicals of interest in the environment.

To develop an analytical method that can monitor targeted/untargeted POPs or POP-like compounds, we chose comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (ToF-MS) because of its superiority to conventional gas chromatography/mass spectrometry (GC/MS), which has been widely used for analysis of POPs. ToF-MS collects full mass spectra typically with better sensitivity than full-scan quadruple-based MS, and GC×GC gives better separation power

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(especially separation of analytes of interest from sample matrix by the second column) and sensitivity than GC alone (*6*). Therefore, GC×GC/ToF-MS is very powerful for simultaneous multiresidue analysis (*7*). However, conventional GC injection techniques typically require extensive cleanup procedures to prevent contamination of the inlet and columns. Skoczyńska et al. successfully analyzed multiple groups of polycyclic aromatic hydrocarbons (PAHs) simultaneously in sediment using GC×GC/ToF-MS but reported instrument troubles from injecting dirty extracts (*8*). Instead, we chose to use direct sample introduction (DSI) which has higher tolerance to dirty extracts than typical splitless injection.

A manual version of DSI was first invented by Amirav and colleagues (*9*). Details of the DSI approach are presented elsewhere (*9*), but essentially, DSI works by injecting the final extract into a disposable microvial in a liner, which is then placed into the inlet. The solvent is evaporated at relatively low temperature, and then the inlet is heated rapidly to introduce the semivolatile chemicals into the GC column. After the GC analysis, the microvial and the liner are removed along with nonvolatile components that would normally contaminate the GC system. DSI helps to reduce sample preparation needs, which expands analytical scope, and also enables large volume injection, which leads to better sensitivity (*10*). Automated commercial versions have been introduced and applications have included multiresidue pesticide analysis in various food matrices (*11, 12*).

Our objective was to develop an approach to analyze a wide scope of chemicals such as multiple groups of known-POPs and untargeted potential contaminants simultaneously in the environment. We used dietary cod liver oil supplement as a test material because fish oil and similar fatty samples are good indicators for bioaccumulative contaminants, and for additional reasons of human dietary intake. To maximize this untargeted screening capability, we assessed different sample cleanup methods with DSI-GC×GC/ToF-MS in terms of the number of analytes identified to select the final sample cleanup method used. During this assessment, we found several untargeted organic chemicals of interest in the fish oils, and in this article, we report/discuss their identification and possible importance.

Experimental Section

The unabridged experimental section including materials, sample preparation, instrumental parameters, data processing, and analytical control is provided in the Supporting Information (SI). A glossary of abbreviations used in this paper is also provided in the SI. Only key information to help follow the results and discussion section is provided below and in Figure 1.

Samples. Dietary cod liver oil supplement (liquid oil) was purchased from an Internet retailer; their source was Norwegian cod liver oil, and the bottle had a label indicating "PCB/heavy metal free." Alaskan sockeye salmon oil supplement was purchased from a local retailer.

Instrumentation and Data Processing. A Pegasus 4D (Leco, St. Joseph, MI) GC×GC/ToF-MS was used with two different column configurations. For both configurations, a Restek (Bellefonte, PA) Siltek deactivated column (5 m, 0.25 mm i.d.) was attached to the inlet as a guard column. The first configuration used a Restek Dioxin2 (60 m, 0.25 mm i.d., 0.25 μm thickness) as the first dimension column (^{1}D) and a Restek PCB (2 m, 0.18 mm, 0.18 μm thickness) as the second dimension column (^{2}D). The other configuration (final method) used a Restek Rtx-5Sil-MS (15 m, 0.25 mm i.d., 0.25 μm film thickness) as the ^{1}D column, and a J&W (Folsom, CA) DB-17MS (2 m, 0.18 mm, 0.18 μm thickness) as the ^{2}D column. The use of the two configurations helped to confirm
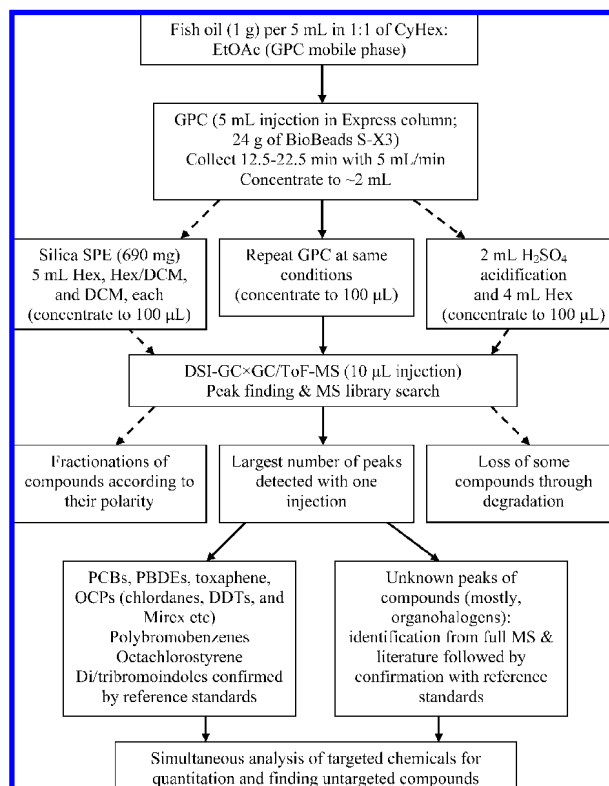

FIGURE 1. Diagram of the qualitative analytical approach used in this study. CyHex, Hex, EtOAc, and DCM signify cyclohexane, hexane, ethyl acetate, and dichloromethane, respectively. Nondashed arrows represent the approach with widest scope.

analyte identities, but ultimately, the second column configuration was chosen because of its shorter analysis time and good resolution of analytes.

A 10 μL injection from a final extract of 100 μL volume was conducted by a Combi-PAL autosampler (Leap Technologies; Carrboro, NC) with the automated DSI accessory (Linex) in combination with an Optic 3 programmable temperature vaporizer (Atas-GL International; Veldhoven, The Netherlands).

Data analysis was conducted with the Leco ChromaTOF (version 3.25) software. Data processing included automatic peak find using MS deconvolution and spectral searching vs the National Institute of Standards and Technology (NIST) 2005 mass spectral library, Agilent pesticide and endocrine disruptor library, and contemporaneously analyzed mass spectra from reference standards. Manual review was made of all integrations and identifications to ensure accuracy of the results.

Results and Discussion

Sample Cleanup Comparison. After the first GPC cleanup step for the 1 g injected sample equivalent, three additional cleanup methods were compared: (1) a second GPC step, (2) silica solid phase extraction (SPE), and (3) acidification with H_2SO_4 (see Figure 1). An extract fraction between 12.5 and 22.5 min in GPC cleanup was chosen to collect a wide scope of chemicals, even though most POPs eluted mainly between 14.5 and 16.5 min. The second GPC step was merely to remove the residual lipid from the first GPC cleanup due to overloading of the column, but this would not be needed in routine analysis if a larger GPC column were used (the capacity of the GPC column used in this study was 0.5 g oil, thus 1 g of cod liver oil equivalent sample required two injections).

A list of peaks found in each extract from the three cleanup methods appears in Table 1. The largest number of peaks

TABLE 1. Compounds Identified in the Extract of Cod Liver Oil Supplement (1 g Equivalent) Using the Three Different Sample Preparation Procedures (The Cod Liver Oil Samples Were Initially Treated by GPC)

peak	compound	ret. time (s) ^1t_R, $^2t_R{}^a$	only GPC	SPE hex	hex/DCM	DCM	H_2SO_4	Id^b	M^+	chemical formula
	~60 PCBs		x	x			x	1		$C_{12}H_nCl_{(10-n)}$
1	BDE-28	1542.5, 2.017	x	x				1	404	$C_{12}H_7Br_3O$
2	BDE-49	1647.5, 2.398	x	x			x	2	482	$C_{12}H_6Br_4O$
3	BDE-47	1668.5, 2.550	x	x			x	1	482	$C_{12}H_6Br_4O$
4	BDE-77 (I.S.)	1717.5, 2.655	x	x			x	1	482	$C_{12}H_6Br_4O$
5	BDE-100	1759.5, 2.934	x	x			x	1	560	$C_{12}H_5Br_5O$
6	BDE-99	1787.5, 2.963	x	x			x	1	560	$C_{12}H_5Br_5O$
7	Hexa-BDE	1840, 0.068	x	x			x	2	638	$C_{12}H_4Br_6O$
8	BDE-154	1857.5, 0.032	x	x			x	1	638	$C_{12}H_4Br_6O$
9	Hexa BDE	1882, 0.484	x	x			x	2	638	$C_{12}H_4Br_6O$
10	BDE-153	1896, 0.087	x	x			x	1	638	$C_{12}H_4Br_6O$
11	Hexachlorobenzene	1217, 0.930	x	x			x	1	282	C_6Cl_6
12	α-Chlordene	1346.5, 1.000	x	x			x	3	336	$C_{10}H_6Cl_6$
13	Heptachlor epoxide	1430.5, 1.380	x			x		1	386	$C_{10}H_5Cl_7O$
14	p,p'-DDMU	1458.5, 1.329	x	x			x	3	282	$C_{14}H_9Cl_3$
15	γ-Chlordane	1458.5, 1.380	x	x	x		x	1	406	$C_{10}H_6Cl_8$
16	α-Chlordane	1472.5, 1.350	x	x	x		x	1	406	$C_{10}H_6Cl_8$
17	trans-Nonachlor	1476, 1.059	x	x			x	1	440	$C_{10}H_5Cl_9$
18	cis-Nonachlor	1546, 1.631	x		x		x	1	440	$C_{10}H_5Cl_9$
19	p,p'-DDE	1497, 1.310	x	x			x	1	316	$C_{14}H_8Cl_4$
20	o,p'-DDD	1507.5, 1.673	x	x	x		x	1	318	$C_{14}H_{10}Cl_4$
21	p,p'-DDD (o,p'-DDT)	1546, 1.690	x	x	x		x	1	318	$C_{14}H_{10}Cl_4$
22	p,p'-DDT	1584.5, 1.688	x	x	x		x	1	352	$C_{14}H_9Cl_5$
23	Dieldrin	1504, 1.651	x		x			1	378	$C_{12}H_8Cl_6O$
24	Mirex	1696.5, 2.330	x	x			x	1	540	$C_{10}Cl_{12}$
25	Toxaphene,^{c}B8−1413^d	1528.5, 1.437	x	x			x	1	410	$C_{10}H_{10}Cl_8$
26	Toxaphene^{c}B8−1414/ B8−1945^e	1584.5, 1.909	x	x			x	1	410	$C_{10}H_{10}Cl_8$
27	Toxaphene,^{c}B8−2229^d	1595, 2.160	x	x			x	1	410	$C_{10}H_{10}Cl_8$
28	Toxaphene,^{c}B9−1679^d	1612.5, 1.838	x	x			x	1	444	$C_{10}H_9Cl_9$
29	Toxaphene,^{c}B9−1025^e	1675.5, 2.630	x	x			x	1	444	$C_{10}H_9Cl_9$
30	Q1 (MBP-Cl$_7$)	1472.5, 1.049	x	x			x	1	384	$C_9H_3Cl_7N_2$
31	MBP-H$_2$Br$_5$	1609, 2.477	x	x				5	536	$C_9H_5Br_5N_2$
32	MBP-HBr$_5$Cl	1626.5, 2.446	x	x				1	570	$C_9H_4Br_5ClN_2$
33	MBP-HBr$_5$Cl	1707, 3.040	x	x				2	570	$C_9H_4Br_5ClN_2$
34	MBP-HBr$_6$	1679, 2.879	x					1	614	$C_9H_4Br_6N_2$
35	MBP-Br$_6$Cl	1766.5, 3.289	x	x			x	1	648	$C_9H_3Br_6ClN_2$
36	MBP-Br$_7$	1812, 0.297	x	x				1	692	$C_9H_3Br_7N_2$
37	DMBP-Br$_4$Cl$_2$	1717.5, 2.942	x	x	x		x	1	540	$C_{10}H_6Br_4Cl_2N_2$
38	DMBP-Br$_6$	1819, 0.442	x		x			1	628	$C_{10}H_6Br_6N_2$
39	MeO-triBDE	1591.5, 2.608	x		x			4	434	$C_{13}H_9Br_3O_2$
40	MeO-triBDE	1602, 1.899	x		x			4	434	$C_{13}H_9Br_3O_2$
41	MeO-triBDE	1623, 2.246	x		x			4	434	$C_{13}H_9Br_3O_2$
42	MeO-chloro-triBDE	1686, 2.459	x		x		x	5	468	$C_{13}H_8Br_3ClO_2$
43	2'-MeO-BDE 68	1714, 2.349	x		x		x	1	512	$C_{13}H_8Br_4O_2$
44	6-MeO-BDE 47	1731.5, 2.800	x		x		x	1	512	$C_{13}H_8Br_4O_2$
45	2,2'-diMeO-BB 80	1717.5, 2.226	x		x		x	1	526	$C_{14}H_{10}Br_4O_2$
46	PBHD (3Br)	1808.5, 3.371	x		x		x	1	464	$C_{16}H_{19}Br_3O$
47	PBHD (4Br)	1941.5, 2.083	x		x		x	1	542	$C_{16}H_{18}Br_4O$
48	Monobromo indole	1178.5, 2.006	x		x	x		2	195	C_8H_6BrN
49	Dibromo indole	1318.5, 1.946	x		x	x		2	273	$C_8H_5Br_2N$
50	Dibromo indole	1399, 2.756	x		x	x		2	273	$C_8H_5Br_2N$
51	Tribromo indole	1504, 2.479	x		x			3	351	$C_8H_4Br_3N$
52	1-Methyl-dibromo-indole	1336, 2.296	x		x			3	287	$C_9H_7Br_2N$
53	2,4,6-Tribromo anisole	1154, 1.114	x	x				1	342	$C_7H_5Br_3O$
54	MHC-1	1465.5, 1.714	x		x			1	396	$C_{10}H_{13}Br_2Cl_3$
55	Octachlorostyrene	1420, 0.890	x	x				1	376	C_8Cl_8
56	Pentabromobenzene	1451.5, 2.416	x	x				5	468	C_6HBr_5
57	Hexabromobenzene	1644, 3.472	x	x			x	1	546	C_6Br_6
58	^{f}Diphenyl methane (4Br and 4OH)	2095.5, 0.094	x		x			4	544	$C_{13}H_8O_4Br_4$
59	Unknown-Br$_3$Cl I	1392, 1.799	x	x	x		x	6	402	$^gC_{10}H_{10}Br_3Cl,^gC_9H_6OBr_3Cl$
60	Unknown-Br$_3$Cl II	1430.5, 1.837	x		x		x	6	402	$^gC_{10}H_{10}Br_3Cl,^gC_9H_6OBr_3Cl$
61	Unknown-Br$_4$Cl	1560, 2.606	x		x		x	6	480	$^gC_{10}H_9Br_4Cl,^gC_9H_5OBr_4Cl$
62	Unknown-Br$_5$Cl	1665, 3.124	x		x		x	6	558	$^gC_{10}H_8Br_5Cl,^gC_9H_4OBr_5Cl$
63	Unknown (6Cl) (m/z 340 375)	1595, 1.857	x		x		x	6	375	$^gC_{12}H_7Cl_6N,^gC_{11}H_3OCl_6N$ $^gC_{10}H_3Cl_6N_3$
64	^{h}Tetrabromobenzene	1245, 1.569	x		x			2	390	$C_6H_2Br_4$

TABLE 1. Continued

peak	compound	ret. time (s)^1t_R, $^2t_R{}^a$	only GPC	SPE hex	hex/DCM	DCM	H_2SO_4	Id^b	M$^+$	chemical formula
65	^{h}Tetrabromobenzene (1,2,4,5)	1280, 2.038	^{h}x	x	^{h}x			1	390	$C_6H_2Br_4$
66	^{h}Tribromophthalic anhydride	1462, 3.083	x		x			5	382	$C_8HBr_3O_3$
67	^{h}Tribromophthalic anhydride	1465.5, 3.161	x		x			5	382	$C_8HBr_3O_3$
68	^{h}Tetrabromophthalic anhydride	1644, 0.441	x		x			1	460	$C_8Br_4O_3$
69	^{h}2-Ethylhexyl tribromobenzoate	1630, 0.869	x		x			5	468	$C_{15}H_{19}Br_3O_2$
70	^{h}2-Ethylhexyl tribromobenzoate	1640.5, 0.843	x		x			5	468	$C_{15}H_{19}Br_3O_2$
71	^{h}2-Ethylhexyl tribromobenzoate	1661.5, 0.788	x		x			5	468	$C_{15}H_{19}Br_3O_2$
72	^{h}2-Ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB)	1784, 1.633	x		x		x	1	546	$C_{15}H_{18}Br_4O_2$
73	^{h}Bis-(2-ethylhexyl)-tribromophthalate	1938, 2.108	x		x		x	5	624	$C_{24}H_{35}Br_5O_4$
74	^{h}Bis-(2-ethylhexyl)-tribromophthalate	1987, 2.908	x		x		x	5	624	$C_{24}H_{35}Br_5O_4$
75	^{h}2-Ethylhexyl,-butyl tetrabromophthalate	1973, 0.210	x		x		x	6	646	$C_{20}H_{26}Br_4O4$
76	^{h}Bis-(2-ethylhexyl)-tetrabromophthalate (TBPH)	2109.5, 1.488	x		x		x	1	702	$C_{24}H_{34}Br_4O_4$
77	Oxybenzone	1420, 1.840	x		x	x		1	228	$C_{14}H_{12}O_3$

^{a} ^1t_R and ^2t_R denote ^{1}D and ^{2}D retention times, respectively, in the shorter column configuration (^{1}D: 15 m Rtx-5Sil-MS with 0.25 mm i.d. and 0.25 μm film thickness, and ^{2}D: 2 m DB-17MS with 0.18 mm and 0.18 μm thickness). ^{b} Id indicates degrees of identification as represented by the numbers 1−6: 1, confirmation by re-analysis and reference standards; 2, MS match with standard but not retention: possibly isomer; 3, MS library match ($\geq$80%); 4, match with MS reported in literature; 5, potential congener based on its MS comparison with other congener standards; 6, presumptive identification of chemical formula based on MS. ^{c} Short terms of individual toxaphene compounds according to Andrews and Vetter ((41)). ^{d} Identified by the correct retention time range on the DB-5 column type, the correct number of chlorines, characteristic features in the mass spectra, and their known abundance in cod liver oil (15, 16). ^{e} Structure tentatively assigned based on the presence of these toxaphenes in cod liver oil and the known elution order on the DB-5 column type (15, 16) ^{f} Tentative structural identification: 2,2′,3,3′-tetrabromo-4,4′5,5′-tetrahydroxy diphenyl methane. ^{g} Tentative molecular formula. ^{h} Detected in procedural blank samples. I.S. represents Internal Standard.

was found in the GPC-only extract followed by a single GC injection. Classical POPs and other known organohalogens were matched with the NIST mass spectral library, and most of them were confirmed by their authentic standards. Unknown compounds were also found, as reported in following sections. Most of the same compounds were similarly detected in the silica SPE extracts, but the compounds were divided into three fractions (hexane, hexane/dichloromethane, and dichloromethane) according to their polarity. This fractionation procedure is commonly used in traditional adsorption cleanup methods, but the fractions could have been combined for routine operations. Another traditional approach is acidification of extracts, but as shown in Table 1, this step lost several of the compounds due to greater chromatographic interferences, analyte degradation, and/or poor recoveries. Numerous hydrocarbons produced from the acidification process interfered with the relatively smaller, more volatile compounds in the chromatograms. Moreover, Covaci et al. reported a degradation problem of halogenated 1,1′-dimethyl-2,2′-bipyrroles (DMBPs) in the presence of sulfuric-acid-impregnated silica used in their fish oil survey study (13). That is perhaps why DMBP-Br$_6$ was not detected after acidification in our study.

This cleanup comparison clearly shows how more cleanup tends to limit analytical scope, but due to the high degree of selectivity and ruggedness attained with DSI-GC×GC/ToF-MS, multiple classes of POPs and untargeted organic compounds in complex extracts can be simultaneously monitored after simple sample preparation. For instance, Stapleton et al. reported a coelution problem between BDE-99 and 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB, peak 72 in Table 1) (14), but this was not an issue in our experiments because the molecular ion was used for identification rather than a Br$^-$ ion, and BDE-99 and TBB were separated in GC×GC. Although GPC-only provided enough cleanup for routine purposes, information gained from silica SPE and

acidification steps was useful to help elucidate possible chemical composition or structures of unknown compounds.

Peak Identification. Table 1 lists the degree of qualitative identification and/or confirmation that was made in the analysis of each detected analyte in the cod liver oil extracts. Presumptive peak identifications from the analyses were conducted using mass spectral libraries (NIST and Agilent pesticide/endocrine disruptor libraries), and then confirmations were made from reanalyses and comparisons with reference standards. Isomers were often identified by their identical mass spectra with their isomeric reference standard, and congeners were tentatively deduced from the spectra of other congeners and isotope ratios of their molecular ions.

Traditional POPs. As expected, well-known POPs such as PCBs and OCPs and POP-like compounds (PBDEs) occurred in the sample. Although the cod liver oil was claimed to be free of PCBs and heavy metals, intense PCB peaks (~60 congeners) were detected, and using PCB and OCP standard mixtures, we were able to confirm the presence of many of those analytes. The main PBDE congeners often found in the environment (BDE-28, 47, 99, 100, 153, and 154) were also detected in the cod liver oil. Based on GC×GC retention times and mass spectra, we confirmed that the toxaphene congeners found in the samples matched those from the toxaphene technical product. By comparing our data with the relative retention times and characteristic mass spectra in the literature (15, 16), we were able to verify that they were the most abundant toxaphene congeners found in the environment (B8-1413, B8-1414/B8-1945, B8-2229, B9-1679, and B9-1025). α-Chlordene and 1-chloro-2,2-*bis*(chlorophenyl)ethene (*p,p'*-DDMU) which is the dechlorinated form of 1,1-dichloro-2,2-*bis*(chlorophenyl)ethane (*p,p'*-DDE) were also identified using the library database.

Halogenated Natural Products. Several halogenated natural products (HNPs) were detected in the extracts: halogenated 1′-methyl-1,2′-bipyrroles (MBPs), DMBPs, meth-

oxylated PBDEs (MeO-PBDEs), polybrominated hexahy-droxanthene derivatives (PBHDs), and polybromoindoles (see Figure S1 in the SI for molecular structures and acronyms). These compounds have structures similar to anthropogenic POPs such as PCBs and PBDEs. In addition, 2,2′-dimethoxy-3,3′5,5′-tetrabromobiphenyl (2,2′-diMeO-BB80), 2,4,6-tri-bromoanisole, and the natural halogenated monoterpene, MHC-1, were found in the sample. Although most of these HNPs have been primarily detected in marine organisms (17−22), it is interesting to see all these HNPs together in the cod liver oil.

Among the MBP congeners (peaks 30−36) we did not have the reference standards to confirm peaks 31 and 33 listed in Table 1, but their mass spectra suggest that they are MBP-H_2Br_5 and MBP-HBr_5Cl. Peak 31's presumptive iden-tification is further supported by literature data (23). Three congeners of MeO-BDE with three bromines each (peaks 39, 40, and 41) were detected as well as the most commonly detected MeO-BDEs (2′-MeO-BDE 68 and 6-MeO-BDE 47) in the cod liver oil (24). Their fragmentation patterns are similar to the MeO-tetra-BDEs but one less bromine in the molecular ions (m/z 434, 436, 438, 440) indicated three bromines. The loss of CH_3Br from the molecule signified an *ortho*-MeO group relative to the diphenyl ether bond (25−27). These MeO-tri-BDEs may be debrominated com-pounds from 2′-MeO-BDE68 and 6-MeO-BDE47 (28). Like-wise, peak 42 is presumably MeO-chloro-tri-BDE because the isotope ratio of the M^+ at m/z 468 represents Br_3Cl, and [M − CH_3Br]$^+$ indicates the *ortho* position of MeO to the diphenyl ether bond (27, 29). Peaks 49 and 50 are likely to be isomers of dibromoindole, in particular 3,4-dibromoindole and 3,6-dibromoindole, respectively, based on comparing their relative retention times with literature information (30). Peak 48 is a monobromoindole isomer of 5H-bromoindole. Peaks 51 (tribromoindole) and 52 (1-methyl-dibromoindole) are tentatively identified based on their high spectral match factors with those compounds in the NIST library.

Tentative Identifications of Other Organohalogen Com-pounds. We tentatively identified peak 58 to be 2,2′,3,3′-tetrabromo-4,4′5,5′-tetrahydroxydiphenylmethane based on its mass spectrum shown in Figure 2. This compound has been found in marine algae (31, 32), and we obtained its archived mass spectrum taken after its extraction from red algae from Jian-Gong Shi (Chinese Academy of Medical Sciences, Beijing, China). Our spectrum matched well with the archived one except for one atom mass unit differences in each of the ion clusters. For example, the M^+ is at m/z 549 in the archived spectrum but at m/z 548 in our MS, and the same difference occurs in the other ion clusters (theoretically, the molecular ion should be m/z 548 for this compound). The greater retention time and broader peak for this compound than for PBHD (4Br) supports that it has higher polarity (presence of four hydroxyl groups), despite their similar molecular weights. In addition, the absence of this compound after the acidification cleanup indicates that this compound might be present in the aqueous phase (greater polarity due to presence of four hydroxyl groups). Other supporting evidence is the high abundance of m/z 388, which probably represents fluorine analogue formed from the loss of two bromines in the *ortho* position to methane in each phenyl and m/z 194 originates from its doubly charged ion.
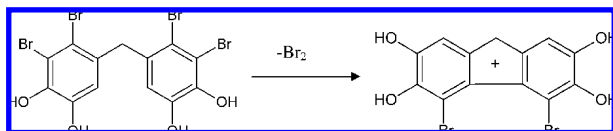
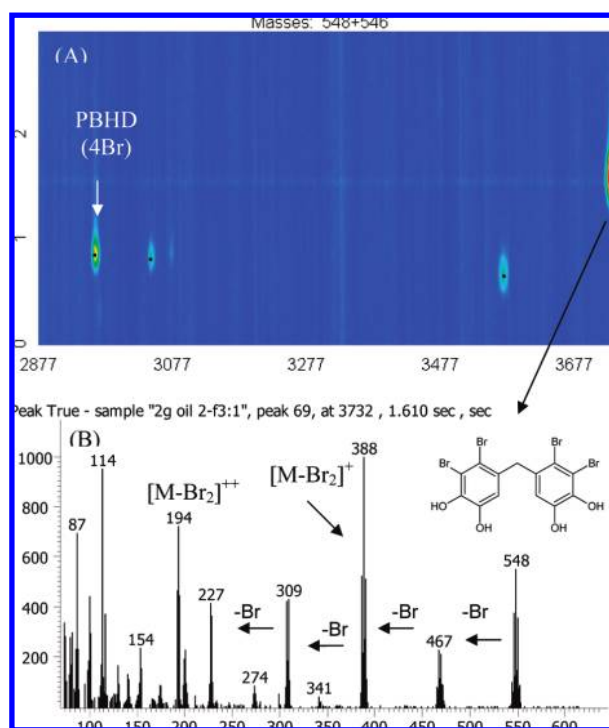Peaks 59−62 seem to contain the same chemical backbone with each other, but a different number of halogens based

FIGURE 2. (A) GC×GC/ToF-MS chromatogram (2D plot) of m/z 548 and m/z 546 indicating PBHD (4Br) and 2,2′,3,3′-tetra-bromo-4,4′5,5′-tetrahydroxy diphenyl methane using the longer ^{1}D column configuration (see details in the Experimental Section). *x*- and *y*-axes represent ^{1}D and ^{2}D retention times in seconds. **(B)** EI mass spectrum of the 2,2′,3,3′-tetrabromo-4,4′5,5′-tetrahydroxy diphenyl methane peak in the cod liver oil.

on their mass spectra (see Figure 3). Peaks 59 and 60 are probably isomers due to their similar spectra. The isotope ratios of their molecular and cluster ions signify that they both contain Br_3Cl. For example, the isotope ratio of m/z 290 [M − BrCl]$^+$, clearly indicates two bromines. Likewise, we were able to interpret that peak 61 has Br_4Cl and peak 62 has Br_5Cl.

Peak 61 (containing Br_4Cl) was detected in the Mediter-ranean sponge S. *scalaris* previously. The mass spectrum from the sponge extract is very similar to ours, but it was assumed to have Br_3Cl_3 in the literature (33). The authors of that report estimated that the compound would have 9 or 10 carbon atoms based on the ratio of carbon isotope ratio of carbon in the M^+. Based on this information, possible elemental compositions for this compound (61) are $C_{10}H_9Br_4Cl$ and $C_9H_5OBr_4Cl$, and the other congeners follow as $C_{10}H_{10}Br_3Cl$/$C_9H_6OBr_3Cl$ (compounds 59 and 60) and $C_{10}H_8Br_5Cl$/$C_9H_4OBr_5Cl$ (compound 62).

There are few suggested molecular structures for these potential elemental compositions in the SciFinder database. Considering the relatively high intensities of the molecular ions and larger fragment ions, these compounds should form stable molecular ions (e.g., they may contain a phenyl ring). They mainly appeared in the Hex:DCM fraction in silica SPE, which suggests that they may be relatively polar and are more likely to have oxygen in its composition. Therefore, brominated phenylchloropropenyl ether (see below) is the closest candidate (CAS 52235-77-5 without a reference) among the possibilities in the SciFinder database, particularly with Br_5Cl.

Among the three mass spectra in Figure 3, [M − Cl]$^+$ in the Unknown-Br_3Cl is more intense than [M − Cl]$^+$ in the others. A possible reason is the more favorable ring closure occurring from abstraction of chlorine from the double bond
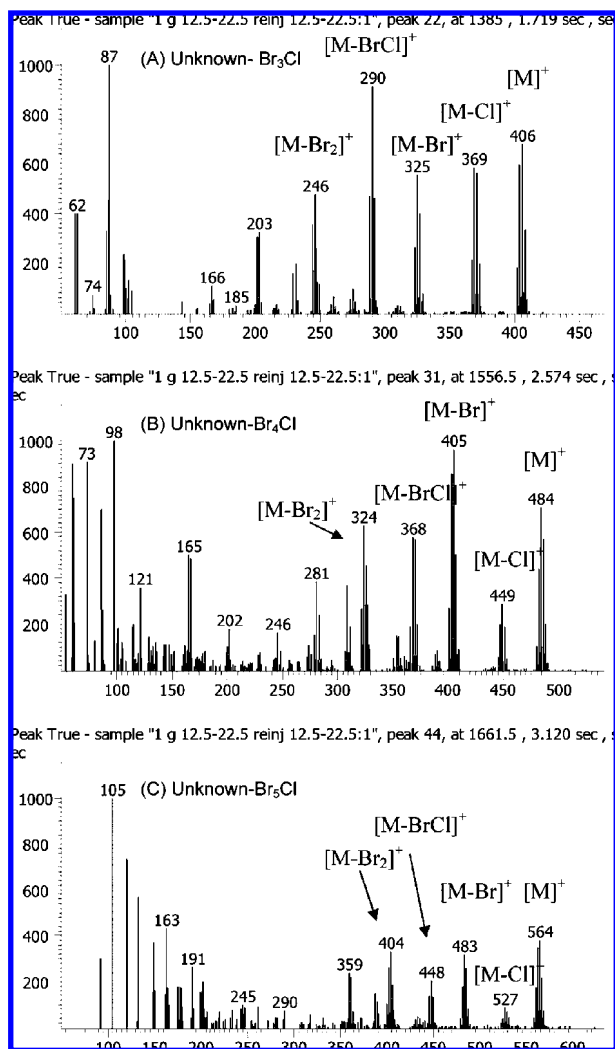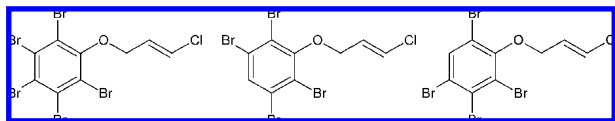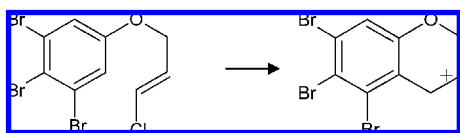
FIGURE 3. EI mass spectra of the unknown peaks 59, 61, and 62 containing (A) Br$_3$Cl, (B) Br$_4$Cl, and (C) Br$_5$Cl. See text for possible chemical structures.

when there is no bromine in ortho positions of oxygen in the phenyl ring.

Loss of chlorine may not be favorable for the Unknown-Br$_5$Cl because fully brominated phenyl may not be favorable to generate the stable ion, ring formation. Therefore, loss of bromine is more favorable in the compound-Br$_5$Cl. The results seem to support our tentative assignments for the unknown peaks, but reference standards are required for confirmation. Another possibility is that these compounds may be related to a brominated compound recently detected in seal blubber, 2-bromoallyl-2,4,6-tribromophenylether (BATE) (34), or an unknown tetrabromo compound (C$_9$H$_6$Br$_4$O) in mussels from central Norway (35).

Peak 63 may have six chlorines based on its isotope ratios of [M]$^+$ and [M − Cl]$^+$ in the mass spectrum shown in Figure 4.
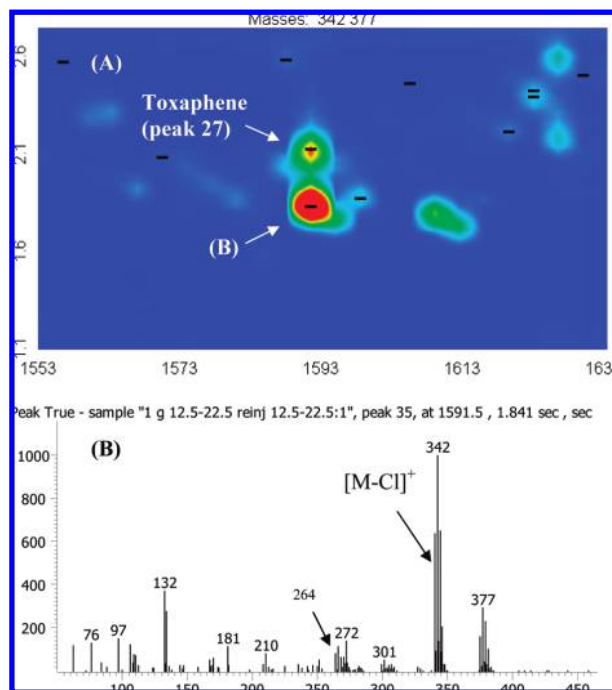
FIGURE 4. (A) GC×GC chromatogram (2D plot) of *m/z* 342 and *m/z* 377 indicating a toxaphene congener (peak 27) and an unknown compound (peak 63) in the cod liver oil using the shorter ^{1}D column configuration (see details in the Experimental Section). *x*- and *y*-axes represent ^{1}D and ^{2}D retention times in seconds; (B) EI mass spectrum of an unknown compound (peak 63) containing six chlorines.

This serves as an excellent example of the power of GC × GC to separate two very similar compounds. If *m/z* 375 is the M$^+$, then the compound's structure should contain an odd number of nitrogen atoms according to the nitrogen rule. The elution of this compound in the Hex:DCM fraction in SPE suggests that it should be relatively polar. However, this requires further investigation for identification.

Oxybenzone. Surprisingly, we discovered and confirmed the nonhalogenated contaminant, oxybenzone (peak 77), in the cod liver oil supplements. Oxybenzone is a common sunscreen agent (36), and it is also used as UV stabilizer in plastic surface coatings for food packaging to prevent polymer or food photodegradation (37). Although its toxicity to humans is low, toxicity studies in animals showed adverse effects upon oral and dermal exposure (38). Oxybenzone has been detected frequently in surface waters, drinking water, wastewater, and even in humans (36, 39, 40). The estimated octanol−water partition coefficient (K_{ow}) of oxybenzone is 3.79 (39), so it is relatively lipophilic and likely to be accumulative in fat. It was detected at 2.7 ng/g in the procedural blanks, while it was determined to be 690 ng/g in the sample extract. Therefore, it is certainly from the cod liver oil sample, but we cannot rule out the possibility of its leaching from the container into the cod liver oil.

Novel Flame Retardants As Laboratory Contaminants (The Unabridged Results and Discussion of This Section Appears in the SI). Peaks 64−76 (tetrabromobenzenes, tri- and tetrabromophthalic anhydrides [PhA-Br$_3$ and PhA-Br$_4$], TBB, *bis*-(2-ethylhexyl) tetrabromophthalate [TBPH] and their debrominated compounds) in Table 1 were detected in both the sample extracts and the blank samples cleaned up by GPC. The intense peaks of these compounds in the GPC blanks (collecting the GPC fraction without making an injection) indicate that they originate from the GPC cleanup step. In addition, detection of the two tetrabromobenzenes in the 1:1 of *n*-hexane/dichloromethane (Hex:DCM) fraction of silica SPE (not in the Hex fraction where penta/hexabro-
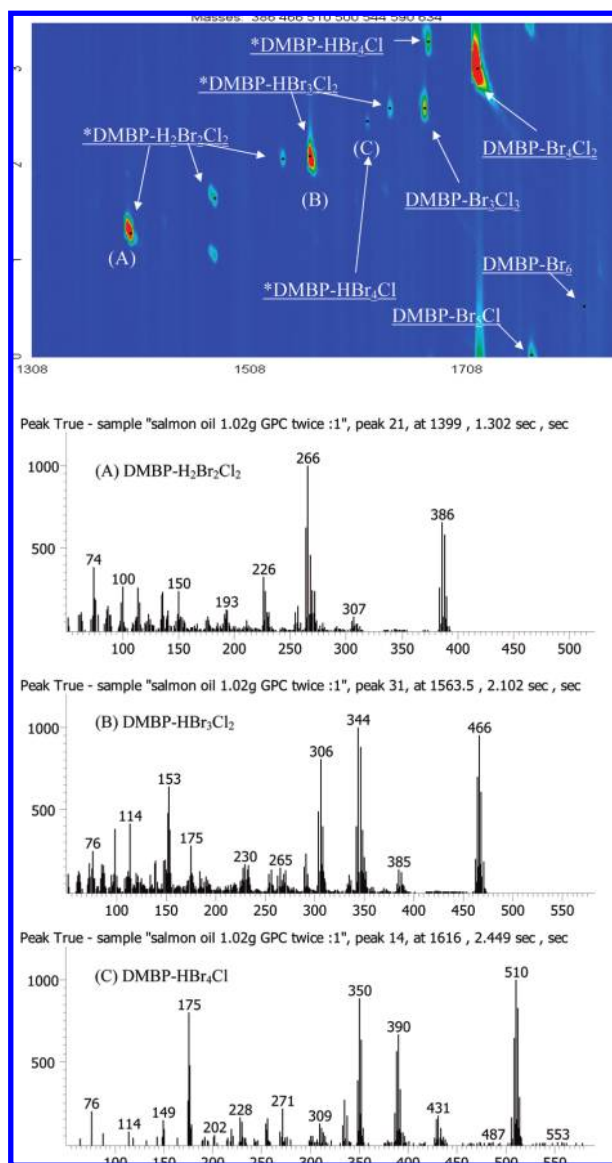
FIGURE 5. GC×GC/ToF-MS chromatogram (2D plot) of m/z 386, 466, 500, 510, 544, 590, and 634 indicating DMBP congeners in the salmon oil using the shorter ¹D column configuration (see details in the Experimental Section). x- and y-axes represent ¹D and ²D retention times in seconds. * indicates newly detected DMBP congeners. EI mass spectra of peaks in the salmon oil potentially identified as (A) DMBP-H$_2$Br$_2$Cl$_2$, (B) DMBP-HBr$_3$Cl$_2$, and (C) DMBP-HBr$_4$Cl.

mobenzenes were detected) indicates that the tetrabromobenzenes do not come from the sample, either. Moreover, phthalic anhydrides are quickly hydrolyzed when moisture is present (5), thus PhA-Br$_3$ and PhA-Br$_4$ are also unlikely to originate from the cod liver oil.

TBB and TBPH were recently detected at high levels in house dust (14), and they are main components of the novel flame retardant Firemaster 550 (FM550). We injected the reference standards (TBB and TBPH), and a technical product, FM550, individually to the DSI-GC×GC/ToF-MS, and confirmed that most of the peaks 64−76 were detected in the chromatograms (Figures S7 and S8). These results show that TBB and TBPH degrade during the hot GC process and produce the several products belonging to 64−76. PhA-Br$_3$ and PhA-Br$_4$ were only detected in the chromatogram of TBPH, which suggests decomposition pathways shown in Figure S9.

The presence of TBB, TBPH, and their related compounds in both the GPC blanks and FM550 indicates that FM550 is a source. Based on our thorough blank experiments in each sample preparation step (see the SI), this contamination occurred during the GPC cleanup step, but we do not know how.

New DMBP Congeners Found in Dietary Salmon Oil Supplement. We applied this analytical approach to another type of fish oil, namely dietary Alaskan sockeye salmon oil. Interestingly, eleven DMBP congeners were detected in the salmon oil while only two DMBP congeners were found in the cod liver oil. Moreover, seven DMBP congeners are reported for the first time (Figure 5). They were tentatively identified by the similarities of their mass spectra to other DMBP reference standards of other congeners (as shown in Figures 5 and S10).

Acknowledgments

Supporting Information Available

This material is available free of charge via the Internet at http://pubs.acs.org.

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ES803486X